

REMARKS

In view of the above amendments and following remarks, reconsideration of the outstanding office action is respectfully requested. Pursuant to 37 CFR § 1.121, attached as Appendix A is a Version with Markings to Show Changes Made.

Asymmetric cell divisions, in which a cell divides to give two daughters with different fates, play an important role in the development of all multicellular organisms. In plants, because there is no cell migration, the regulation of asymmetric cell divisions is of heightened importance in determining organ morphology. In contrast to animal embryogenesis, most plant organs are not formed during embryogenesis. Rather, cells that form the apical meristems are set aside at the shoot and root poles. These reservoirs of stem cells are considered to be the source of all post-embryonic organ development in plants. A fundamental question in developmental biology is how meristems function to generate plant organs.

Root organization is established during embryogenesis. This organization is propagated during postembryonic development by the root meristem. Following germination, the development of the postembryonic root is a continuous process, wherein a series of initials or stem cells continuously divide to perpetuate the pattern established in the embryonic root.

Due to the organization of the *Arabidopsis* root, it is possible to follow the fate of cells from the meristem to maturity and identify the progenitors of each cell type. The *Arabidopsis* root is a relatively simple and well characterized organ. The radial organization of the mature tissues in the *Arabidopsis* root has been likened to tree rings with the epidermis, cortex, endodermis, and pericycle forming radially symmetric cell layers that surround the vascular cylinder. These mature tissues are derived from four sets of stem cells or initials: (i) the columella root cap initial; (ii) the pericycle/vascular initial; (iii) the epidermal/lateral root cap initial; and (iv) the cortex/endodermal initial. It has been shown that these initials undergo asymmetric divisions. The cortex/endodermal initial, for example, first divides anticlinally (in a transverse orientation). This asymmetric division produces another initial and a daughter cell. The daughter cell, in turn, expands and then divides periclinally (in the longitudinal orientation). This second asymmetric division produces the progenitors of the endodermis and the cortex cell lineages.

Furthermore, root radial organization in *Arabidopsis* is produced by three distinct developmental strategies. First, primary roots employ stem cells which initially undergo asymmetric divisions first to regenerate themselves and then to generate the cell lineages of the root. Second, in the embryo, sequential asymmetric divisions subdivide pre-existing tissue to form the cell layers of the embryonic root. Finally, lateral roots are formed by a strategy of cell proliferation that originates in differentiated tissues. Remarkably, within a given species, all three strategies result in roots with a nearly identical radial organization.

The root organization of *Zea mays* (maize), which is a very well characterized member of the grass family, is far more complex than the root organization in *Arabidopsis*. The root system of maize consists of primary, embryonic, lateral, seminal lateral, and adventitious roots. Both primary and seminal lateral roots are formed during embryogenesis, where the primary root is the first root to emerge during germination, followed by the seminal lateral roots formed at the scutellar nodal region. Both crown and prop roots which develop post-embryonically are shoot-borne roots, often termed “adventitious.” However, since these roots are part of the normal development of the plant, they are not, strictly speaking, adventitious roots, which are typically formed as a result of injury or hormone treatment. Crown roots, representing the major roots of the mature plant, are formed at consecutive early nodes of the stem beginning with the coleoptilar nodes. Later in development, brace or prop roots emerge from nodes above the soil level.

Currently, there are two notably different types of organization of root apical meristems: an open and a closed meristem. In an “open” meristem, the cell files of the mature tissues cannot be traced with much confidence to distinct initials, and the incipient tissues do not appear to have discrete boundary walls between the root proper and the root cap. Therefore, the interpretation of the organization of the open meristem has been problematic. In a “closed” meristem, however, since files of cells converge onto a pole at the root apex, it is easy to identify discrete layers in median longitudinal sections.

Both *Arabidopsis* and maize roots show characteristics of the closed meristem. However, there are important differences. In maize roots, the root apical meristem consists of three independent layers of initials. One gives rise to the stele, the second gives rise to epidermis, cortex, and endodermis, and the third generates the root cap. In the *Arabidopsis* root apical meristem, the epidermis shares a common initial with the lateral root cap.

Primary organization of the root apical meristem in maize occurs during embryogenesis, as in *Arabidopsis*. There are three main phases in embryo development in maize. As in *Arabidopsis*, the very first division of the zygote establishes the initial asymmetry of the embryo. However, unlike *Arabidopsis*, embryonic development in maize is characterized by rather irregular cell divisions. During the first phase, the apical-basal asymmetry of the embryo is established and the embryo is regionalized into suspensor and embryo proper. During the second phase, radial asymmetry appears and the embryonic axis and meristems are established. Finally, during the third phase, vegetative structures such as embryonic roots and leaves are elaborated.

The quiescent center (QC) of root apical meristems of angiosperms is a population of mitotically inactive cells. In the QC of the primary root of maize, for example, the average duration of a mitotic cycle is about 200 hours compared with only 12 hours in the cells just below the QC and 28 hours in the cells just above the QC. Moreover, there are also reductions in the rates of synthesis of DNA and protein, and corresponding reductions in the amounts of DNA and RNA per cell.

Although the precise role of the QC has remained speculative, it is generally accepted that cells within the QC are undifferentiated and, other than the anatomical pattern of cell files, lacking in radial pattern information. This theory has been supported by ablation studies performed in *Arabidopsis*, where complete laser ablation of the four central cells in the *Arabidopsis* QC led to subsequent restoration of the QC by cells of the stele. Furthermore, laser ablation of only one or two cells in the QC resulted in differentiation of surrounding initial cells. Analysis of the *hobbit* mutants further supports these observations. In the *hobbit* mutants, there is no functional QC, leading all cortex initials to divide into cortex and endodermis during embryogenesis. Taken together, it is suggested that the QC suppresses differentiation of surrounding initials in the range of a single cell.

In maize, on which the contemporary view of the role of the QC is based, surgical and tissue culture systems were developed to study the organization process of root apical meristems. Following removal of the QC, the remaining root regenerates a new root tip. This process appears to involve *de novo* organization of the QC and the apical meristem. In addition, the excised QC itself is capable of generating a new root. This suggests that there is indeed sufficient radial pattern information in the QC to allow the regeneration of more or less intact roots.

Mutations that disrupt the asymmetric divisions of the cortex/endodermal initial have been identified and characterized. *short-root (shr)* and *scarecrow (scr)* mutants are missing a cell layer between the epidermis and the pericycle. In both types of mutants, the cortex/endodermal initial divides anticlinally, but the subsequent periclinal division that increases the number of cell layers does not take place. The defect is first apparent in the embryo, and it extends throughout the entire embryonic axis, which includes the embryonic root and hypocotyl. This is true also for other radial organization mutants characterized to date, suggesting that radial patterning that occurs during embryonic development may influence the post-embryonic pattern generated by the meristematic initials.

Characterization of the mutant cell layer in *shr* indicated that two endodermal-specific markers were absent. This provided evidence that the wild-type *SHR* gene may be involved in the specification of endodermis identity.

In plants, the capacity for gravitropism has been correlated with the presence of amyloplast sedimentation. Amyloplast sedimentation only occurs in cells in specific locations at distinct developmental stages. That is, when and where sedimentation occurs is precisely regulated. In roots, amyloplast sedimentation only occurs in the central (columella) cells of the rootcap; as these cells mature into peripheral cap cells, the amyloplasts no longer sediment. In stems of many plants, including *Arabidopsis*, amyloplast sedimentation occurs in the starch sheath (endodermis) especially in elongating regions of the stem.

Gravitropic mutants have been studied for evidence that proves the role of amyloplast sedimentation in gravity sensing. However, many gravitropic mutations affect downstream events such as auxin sensitivity or metabolism. Other mutations seem to affect gene products that process information from gravity sensing. For example, the lazy mutants of higher plants and comparable mutants in mosses can clearly sense and respond to gravity, but the mutations reverse the normal polarity of the gravitropic response. Other mutations appear to affect gravitropism of specific organs. For example, *sgr* mutants have defective shoot gravitropism.

In response to the restriction requirement, applicants hereby confirm the election of Group I (i.e., claims 29-41, 44, and 45). Non-elected claims 42, 43, and 46 have been canceled without prejudice to them being pursued in a divisional application.

The objection to claims 36 and 38 for reading on non-elected material is obviated in view of the amendments to claims 36 and 38.

The rejection of claims 29-41, 44, and 45 under 35 U.S.C. § 101 for lack of a specific asserted utility or a well established utility is respectfully traversed.

The specification states that the claimed invention can be used to “improve agronomically valuable plants” (page 24, lines 28-29). In particular, the isolated nucleic acid molecule of the present invention is “a gene involved in the regulation of a specific asymmetric division, in controlling gravitropic response in aerial structures, and in controlling pattern formation in roots” (page 24, lines 31-34). This isolated nucleic acid molecule may be used in constructing DNA and expression vectors (i.e., gene constructs), which are described in the specification as being useful “to alter the root and/or stem structure, and the gravitropism of aerial structures of transgenic plants” (page 26, lines 3-4). Regarding transgenic plants overexpressing the SCARECROW protein, the specification states the following:

Since *SCR* regulates root cell divisions, overexpression of *SCR* can be used to increase division of certain cells in roots and thereby form thicker and stronger roots. Thicker and stronger roots are beneficial in preventing plant lodging.

* * *

Since *SCR* affects gravitropism of aerial structures, overexpression of *SCR* may be used to develop “straighter” transgenic plants that are less susceptible to lodging.

(page 26, lines 5-9 and 12-15).

The U.S. Patent and Trademark Office (“USPTO”) appears to base its rejection on its view that the applicants have not presented data supporting the claims of increased cell division in the root and hypocotyl with a concomitant alteration of the hypocotyl gravitropic response when *SCR* is overexpressed in plants. Instead, the USPTO asserts that the data presented thus far only supports the use of the *SCR* gene to complement *scr* mutant plants to produce a wild-type plant.

To overcome this objection, applicants are submitting herewith the Declaration of Philip N. Benfey Under 37 C.F.R. § 1.132 (“Benfey Declaration”), which includes additional experimental data supporting the utility of the present invention. Transgenic *Arabidopsis thaliana* plants overexpressing the *Zea mays SCARECROW* gene (*ZmSCR*) of the present invention were produced and analyzed (Benfey Declaration ¶¶ 7-8). The results from these experiments show that plants transformed with *ZmSCR* exhibit increased cell division in root and hypocotyl tissue, resulting in transgenic plants having thicker roots, straighter shoots, and less susceptibility to lodging than non-transgenic plants (Benfey Declaration ¶ 8). In view of these experimental results, applicants respectfully

submit that the rejection of claims 29-41, 44, and 45 under 35 U.S.C. § 101 is improper and should be withdrawn.

The rejection of claims 29-41, 44, and 45 under 35 U.S.C. § 112 (1st para.) for failure to support a specific utility is respectfully traversed. Applicants respectfully submit that this rejection should be withdrawn for substantially the same reasons provided above in response to the rejection under 35 U.S.C. § 101.

The rejection of claims 36-39 under 35 U.S.C. § 112 (1st para.) for lack of enablement is respectfully traversed. One of ordinary skill in the art would be fully able to generate the claimed transgenic plants, particularly in view of Example 3 (page 95, line 1 to page 97, line 5) of the present application. Moreover, as discussed above, the Benfey Declaration demonstrates that the claimed utility can be achieved with the present invention. Thus, the rejection for lack of enablement is improper and should be withdrawn.

The rejection of claims 36, 38, and 39 under 35 U.S.C. § 112 (2nd para.) for indefiniteness is respectfully traversed in view of the above amendments.

In view of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,



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Date <u>December 5, 2002</u>	<u>Jo Ann Whalen</u> Jo Ann Whalen

APPENDIX A
Version With Markings to Show Changes Made

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In reference to the amendments made herein to claims 36, 38, and 39, additions appear as underlined text, while deletions appear as bracketed text, as indicated below:

In The Claims:

36. (Amended) A plant genetically-engineered to overexpress [or underexpress] a SCARECROW protein or polypeptide, said protein or polypeptide being encoded by the nucleic acid molecule of claim 29 or 30, wherein cell division in the plant is increased, resulting in thicker roots and/or straighter stems than a non-genetically engineered plant [modified, and root and/or stem development is altered].

38. (Amended) A plant genetically-engineered to overexpress [or underexpress] a SCARECROW protein or polypeptide comprising SEQ ID NO: 96, wherein the [gravitropism of the] plant's stem or hypocotyl [is altered] exhibits stronger directional growth away from a gravity vector than non-genetically engineered plants.

39. (Amended) The plant of claim 38 that is less susceptible to lodging than a [wild-type] non-genetically engineered plant.